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# Supplementation of conjugated linoleic acid in dairy cows reduces endogenous glucose production during early lactation[1]

**Kristin Hötger,*[2] Harald M. Hammon,*[2] Claudia Weber,* Solvig Görs,* Arnulf Tröscher,† Rupert M. Bruckmaier,‡ and Cornelia C. Metges*[3]**
*Department of Nutritional Physiology 'Oskar Kellner', Leibniz Institute for Farm Animal Biology, 18196 Dummerstorf, Germany
†BASF SE, 67117 Limburgerhof, Germany
‡Veterinary Physiology, Vetsuisse Faculty, University of Bern, 3001 Bern, Switzerland

## ABSTRACT

*Trans*-10,*cis*-12 conjugated linoleic acid (CLA) supplementation causes milk fat depression in dairy cows, but CLA effects on glucose metabolism are not clear. The objective of the study was to investigate glucose metabolism, especially endogenous glucose production (eGP) and glucose oxidation (GOx), as well as hepatic genes involved in endogenous glucose production in Holstein cows supplemented either with 50 g of rumen-protected CLA (9% *trans*-10,*cis*-12 and 10% *cis*-9,*trans*-11; CLA; n = 10) or 50 g of control fat (24% C18:2; Ctrl; n = 10) from wk 2 before parturition to wk 9 of lactation. Animal performance data were recorded and blood metabolites and hormones were taken weekly from 2 wk before to 12 wk after parturition. During wk 3 and 9 after parturition, glucose tolerance tests were performed and eGP and GOx were measured by [U-$^{13}$C] glucose infusion. Liver biopsies were taken at the same time to measure total fat and glycogen concentrations and gene expression of pyruvate carboxylase, cytosolic phosphoenolpyruvate carboxykinase, glucose-6-phosphatase, and carnitine palmitoyl-transferase 1. Conjugated linoleic acid feeding reduced milk fat, but increased milk lactose output; milk yield was higher starting 5 wk after parturition in CLA-fed cows than in Ctrl-fed cows. Energy balance was more negative during CLA supplementation, and plasma concentrations of glucose were higher immediately after calving in CLA-fed cows. Conjugated linoleic acid supplementation did not affect insulin release during glucose tolerance tests, but reduced eGP in wk 3, and eGP and GOx increased with time after parturition. Hepatic gene expression of cytosolic phosphoenolpyruvate carboxykinase tended to be lower in CLA-fed cows than in Ctrl-fed cows. In spite of lower eGP in CLA-fed cows, lactose output and plasma glucose concentrations were greater in CLA-fed cows than in Ctrl-fed cows. This suggests a CLA-related glucose sparing effect most likely due to lower glucose utilization for milk fat synthesis and probably because of a more efficient whole-body energy utilization in CLA-fed cows.

**Key words:** conjugated linoleic acid, endogenous glucose production, transition period

## INTRODUCTION

High-yielding dairy cows are not able to compensate completely for the energy lost due to milk production during early lactation by a sufficient increase of feed intake (Bell and Bauman, 1997; Drackley et al., 2001; Kokkonen et al., 2005). This leads to the mobilization of body reserves resulting in a negative energy balance that may be associated to health problems. In turn, this can negatively affect animal welfare and profitability of milk production (Bauman, 2000; Drackley et al., 2001; Eastridge, 2006). One possibility to reduce milk energy output in dairy cows is the decrease of milk fat content by feeding rumen-protected conjugated linoleic acid (**CLA**), in particular the *trans*-10,*cis*-12 CLA isomer (Baumgard et al., 2000; Bauman et al., 2008). Reports on CLA-induced alterations of macronutrient and energy metabolism in cows are inconsistent, and CLA effects may depend on the dietary status and the stage of lactation of the investigated cows (Bauman and Griinari, 2003; Selberg et al., 2004; Castañeda-Gutiérrez et al., 2005; Odens et al., 2007). Concerning glucose metabolism, CLA elevated plasma glucose concentrations in one study (Odens et al., 2007), but, in general, investigations on glucose metabolism with respect to CLA supplementation in dairy cows are limited to glucose concentrations in blood plasma and insulin responses (Bauman et al., 2008). However, it is unknown if CLA supplementation in dairy cows also changes glucose turnover.

Availability of glucose in the mammary gland is essential for high lactose production and, thus, milk production in dairy cows (Drackley et al., 2001; Brockman,

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[2]Both authors contributed equally to this paper.
[3]Corresponding author: metges@fbn-dummerstorf.de

2005) because lactose is the major osmoregulator for mammary uptake of water (Linzell, 1972; Rigout et al., 2002). In ruminants, very little glucose originates from net portal absorption, and endogenous glucose production (**eGP**); that is, the sum of gluconeogenesis and glycogenolysis, provides most of the glucose for milk production (Danfaer, 1994; Bell and Bauman, 1997; Brockman, 2005; Aschenbach et al., 2010). Therefore, hepatic glucose production strongly increases after calving in dairy cows to provide glucose (Danfaer, 1994; Bell and Bauman, 1997; Brockman, 2005; Aschenbach et al., 2010). However, it is not known if CLA supplementation affects eGP as well as glucose oxidation (**GOx**) in high-yielding dairy cows during early lactation. As feeding *trans*-10,*cis*-12 CLA reduces milk fat synthesis, and glucose is needed for milk fat synthesis (Grummer and Carroll, 1991; Bauman and Griinari, 2003), we tested the hypothesis that CLA supplementation may decrease eGP and affect hepatic gene expression of enzymes involved in eGP and hepatic FA oxidation to explain lower glucose use due to reduced milk fat synthesis. Therefore, the objective of this study was to quantify in vivo eGP and GOx, as well as hepatic mRNA abundance of enzymes involved in gluconeogenesis and FA oxidation of CLA-supplemented dairy cows.

## MATERIALS AND METHODS

### Animals and Feeding

All experimental procedures were carried out according to German animal protection law and approved by the relevant authorities of the state Mecklenburg-Vorpommern, Germany. Twenty Holstein cows with comparable milk production (first lactation >9,000 kg of milk in 305 d) from the research herd of the Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, or purchased from a local farm, were kept in a tie stall and were randomly allocated to 2 groups before the beginning of their second lactation.

Cows were fed ad libitum a TMR supplemented with either with 50 g/d of Lutrell pure (BASF, Ludwigshafen, Germany) containing 9.3% *trans*-10,*cis*-12 and 10.3% *cis*-9,*trans*-11 CLA isomers (CLA; n = 10), or 50 g/d of control fat (BASF; **Ctrl**; n = 10; Table 1). Both fat types were provided in a rumen-protected form. Conjugated linoleic acid supplementation lasted from 2 wk before estimated calving date to wk 9 of lactation (supplementation period). From wk 10 to 12 in both groups, fat supplementation was removed from the ration (depletion period); the experiment was terminated at wk 12 of lactation. Cows were investigated in 5 blocks consisting of 4 cows in each block (2 Ctrl and 2 CLA cows) from July 2009 to August 2010. Cows did not suffer from clinical diseases such as metritis, milk fiber, ketosis, or displaced abomasum. When mastitis occurred, cows were treated with antibiotics and milk data during clinical mastitis were removed from data analyses.

**Table 1.** Fatty acid composition of conjugated linoleic acid (CLA) and control (Ctrl) supplements[1]

| Fatty acid (%) | CLA | Ctrl |
|---|---|---|
| C14:0 | 0.24 | 0.28 |
| C15:0 | <0.1 | <0.1 |
| C16:0 | 11.11 | 11.55 |
| C16:1 | <0.1 | <0.1 |
| C18:0 | 53.34 | 53.50 |
| C18:1 *cis*-9 | 10.43 | 10.12 |
| C18:2 *cis*-9,*cis*-12 | 0.67 | 23.52 |
| C18:2 *trans*-10,*cis*-12 CLA | 9.26 | <0.1 |
| C18:2 *cis*-9,*trans*-11 CLA | 10.30 | <0.1 |
| C18:3 *cis*-9,*cis*-12,*cis*-15 | <0.1 | <0.1 |
| C20:0 | 0.53 | 0.55 |
| C22:0 | 0.49 | 0.47 |
| C24:0 | <0.1 | <0.1 |
| Others | 3.62 | — |

[1]CLA-supplemented cows were fed ad libitum TMR (Table 2) supplemented with 50 g/d of Lutrell pure (BASF, Ludwigshafen, Germany) from 14 d before calving to 63 DIM. Ctrl cows were fed ad libitum TMR (Table 2) supplemented with 50 g/d of control fat (rumen-protected sunflower oil from BASF) from 14 d before calving to 63 DIM.

Cows had free access to water. Individual feed intake was recorded daily. Feed samples (TMR, corn and grass silage) were pooled weekly, stored at −20°C until analyzed according to Naumann and Bassler (2004) at the Agricultural Faculty of the University of Rostock and at the Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA, Rostock, Germany). Ingredients and chemical composition for the close-up (wk 2–0 before parturition) and lactation diet (wk 1–12 of lactation) are shown in Table 2. Cows were milked twice daily and milk samples were taken once weekly for measurement of fat, lactose, and protein in milk at the Landeskontrollverband für Leistungs- und Qualitätsprüfung Mecklenburg-Vorpommern e.V. (Güstrow, Germany). In milk samples collected in wk 3 and 9 of lactation, FA pattern was determined at the Lehrstuhl für Tierzucht, Technische Universität München (Kranzberg, Germany) as described (Sigl et al., 2010). Energy-corrected milk and energy balance were calculated as described (Hammon et al., 2009). Body weight, BCS, and back fat thickness (**BFT**) were recorded weekly (Duske et al., 2009).

### Metabolites and Hormones

Blood samples were taken by jugular venipuncture (Vacuette, Greiner Bio One International, Kremsmünster, Austria) once weekly (day relative to calving ± 1 d tolerance) after the morning milking and before cows